Claims

1. A method for the joint and in each case specific detection of a mycobacterial infection, of the *Mycobacterium tuberculosis* complex and/or of *Mycobacterium avium* in clinical material comprising the steps of

a) extraction of microbial DNA from clinical material,

b) amplification of at least one fragment of the 16S rRNA gene from the extracted DNA by means of a primer pair including the nucleotide sequences SEQ ID NO: 1/SEQ ID NO: 5 or by means of two primer pairs, where one primer pair includes the nucleotide sequences SEQ ID NO: 2/SEQ ID NO: 3 and the other primer pair includes the nucleotide sequences SEQ ID NO: 4/SEQ ID NO: 5,

c) detection of the genus-specific region of the amplified 16S rRNA fragment of mycobacteria by means of a pair of

labeled hybridization probes, where the pair includes the nucleotide sequences SEQ ID NO: 10/SEQ ID NO: 11,

d) detection of the species-specific region of the amplified 16S rRNA fragment of mycobacteria by means of a pair of labeled hybridization probes, where the pair includes the nucleotide sequences SEQ ID NO: 6/SEQ ID NO: 7 or the complementary sequences thereof for detecting the *Mycobacterium tuberculosis* complex, and where the pair includes the nucleotide sequences of SEQ ID NO: 8/SEQ ID NO: 9 or the complementary sequences thereof for detecting *Mycobacterium avium*, and

e) where the joint, in each case specific detection of mycobacteria and of the *Mycobacterium tuberculosis* complex and/or of *Mycobacterium avium* takes place during the detection as in steps c) and d) by means of melting curve analysis.

2. The method as claimed in Claim 1, where the extracted microbial DNA is mixed in step a) with at least one artificial plasmid, comprising at least one sequence selected from SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO:16 and SEQ ID NO: 17, as internal standard, and a melting curve analysis is carried out for specific detection of the amplified 16S rRNA fragments of mycobacteria and of the modified 16S rRNA fragments of the artificial plasmid.

3. The method as claimed in Claim 1, where the extracted microbial DNA is divided and a first portion thereof is further treated in the method specified in steps a) to e) of Claim 1, and a second portion is subjected to a parallel method comprising the steps of

a') mixing of the extracted microbial DNA with at least one artificial plasmid, comprising at least one sequence selected from SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17, as internal standard,

b') amplification of the 16S rRNA fragments by means of at least one primer pair selected from the group including the nucleotide sequence pairs SEQ ID NO: 1/SEQ ID NO: 5 and SEQ ID NO: 4/SEQ ID NO: 5,

c') detection of the amplified 16S rRNA fragments by means of a pair of labeled hybridization probes which hybridize with the genus-specific region III of the 16S rRNA fragment of mycobacteria, where the pair includes the nucleotide sequences SEQ ID NO: 10/SEQ ID NO: 11 or the complementary sequences thereof, and

d') where a melting curve analysis takes place for the specific detection of the amplified 16S rRNA fragments of mycobacteria and of the modified 16S rRNA fragments of the at least one artificial plasmid during the detection as in step c').

4. The method as claimed in any of the preceding claims, where the amplification of the gene fragments is carried out by means of the polymerase chain reaction (PCR).

5. The method as claimed in claim 4, where the polymerase chain reaction (PCR) is carried out as real-time PCR, preferably by means of the LightCyclerTM system.

6. The method as claimed in any of the preceding claims, where the detection takes place during or after the amplification of the 16S rRNA fragments.

7. The method as claimed in any of the preceding claims, where the detection takes place by means of real-time PCR, particularly preferably by means of the LightCyclerTM system.

8. The method as claimed in any of the preceding claims, where the detection is carried out by means of fluorescence detection, and where the labeled hybridization probe pairs are configured as fluorescence resonance energy transfer pair.

9. The method as claimed in any of the preceding claims, where the melting curve analysis takes place following the amplification of the 16S rRNA fragments by means of real-time PCR, preferably by means of the LightCyclerTM system.

10. The method as claimed in Claim 8 or 9, where the detection is carried out as quantitative measurement.

11. The method as claimed in any of the preceding claims, where the clinical material is selected from the group of clinical samples consisting of sputum, bronchial lavage, gastric juice, urine, stool, CSF, bond marrow, blood and biopsies.

12. An oligonucleotide primer pair with SEQ ID NO: 2 and SEQ ID NO: 3.

13. An oligonucleotide primer with SEQ ID NO: 4.

14. An oligonucleotide hybridization probe pair with SEQ ID NO: 6 and SEQ ID NO: 7 or with the complementary sequences thereof.

15. An oligonucleotide hybridization probe pair with SEQ ID NO: 8 and SEQ ID NO: 9 or with the complementary sequences thereof.

16. An oligonucleotide hybridization probe pair with SEQ ID NO: 10 and SEQ ID NO: 11 or with the complementary sequences thereof.

17. An artificial plasmid which can be employed as internal control of the amplification and of the detection of 16S rRNA fragments of mycobacteria, comprising at least

one sequence selected from SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16 and SEQ ID NO: 17.

18. A diagnostic kit for the specific detection of a mycobacterial infection and of the Mycobacterium tuberculosis complex and of Mycobacterium avium in clinical material by the method as claimed in any of Claims 1 to 11 including:

a) at least one polymerase,

b) at least one primer pair with SEQ ID NO: 2 and SEQ ID NO: 3,

c) at least one primer pair comprising a primer with SEQ ID NO: 4 which amplifies the genus-specific region III of mycobacteria.

d) a hybridization probe pair with SEQ ID NO: 10 and SEQ ID NO: 11 or with the complementary sequences thereof for the detection of the genus-specific region III,

e) at least one hybridization probe pair with SEQ ID NO: 6 and SEQ ID NO: 7 or the complementary sequences thereof and/or with SEQ ID NO: 8 and SEQ ID NO: 9 or the complementary sequences thereof for the detection of the species-specific regions.

19. A diagnostic kit as claimed in Claim 18, comprising an artificial plasmid as claimed in Claim 17.